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# COPPER OXIDE NANOPARTICLE-BASED SEED PRIMING ENHANCES GERMINATION, GROWTH, AND YIELD IN OKRA (*Abelmoschus esculentus* L.)

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**Abstract:** Poor seed germination and weak early vigor limit growth and yield of okra (*Abelmoschus esculentus* L.). Seed priming is a promising strategy for improving crop establishment, and nanoparticle-based priming may enhance nutrient delivery compared to ionic sources. In this study, copper oxide nanoparticles (CuO NPs) were green-synthesized using radish (*Raphanus sativus* L.) leaf extract and characterized by FTIR, FESEM–EDS, and zeta potential analysis. The CuO NPs exhibited near-spherical morphology (150–250 nm), confirmed Cu–O bonding, and moderate colloidal stability (–23.21 mV). Seed priming did not significantly affect germination percentage; however, seedling growth responses were strongly treatment dependent. CuO NP priming at 25–50 ppm significantly enhanced root length and fresh biomass, while CuSO<sub>4</sub> treatments caused growth inhibition. Pot experiments showed that CuO NP priming significantly increased plant height, pod number, pod length, and total yield, with maximum yield recorded at 50 ppm. Strong positive correlations were observed among vegetative growth, pod traits, and yield. In conclusion, green-synthesized CuO NPs applied through seed priming were more effective than ionic copper in enhancing okra growth and productivity, highlighting their potential as a nano-enabled strategy for sustainable crop improvement.

**Keywords:** Copper oxide nanoparticles; Green synthesis; Seed priming; Okra (*A. esculentus* L.); Nanofertilizer; Sustainable agriculture

## 1. Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important warm-season vegetable cultivated extensively across tropical and subtropical regions, where it contributes significantly to food security, farm income, and nutritional quality due to its high fiber, vitamin, and mineral content. Global okra productivity, however, remains highly variable and is strongly constrained by poor seed germination, non-uniform seedling establishment, and weak early vigor, particularly under suboptimal environmental conditions [21, 37]. These constraints are largely attributed to inherent seed dormancy, hard seed coats, and sensitivity of early developmental stages to abiotic stress, which together reduce stand uniformity and final yield potential [7, 23]. Seed priming has emerged as a widely adopted, low-input agronomic strategy to overcome such limitations by partially hydrating seeds to activate metabolic processes while preventing radicle emergence [40]. Numerous studies have demonstrated that priming enhances germination rate, synchrony, seedling vigor, and stress tolerance by improving membrane integrity, enzyme activation, and reserve mobilization [5, 14]. In okra specifically, conventional priming techniques such as hydro-priming, halo-priming, and osmopriming have been shown to improve germination and early growth under salinity, drought, and temperature stress, although their effects are often transient and highly dependent on environmental conditions [21, 23].

Recent advances in agricultural nanotechnology have led to the development of nano-priming, a next-generation seed enhancement approach in which seeds are treated with engineered nanoparticles capable of modulating water uptake, redox balance, enzymatic activity, and stress-responsive signaling pathways during germination and early seedling growth [27, 40]. Metal and metal oxide nanoparticles (MONPs) are of particular interest due to their high surface area, reactivity, and ability to deliver micronutrients in a controlled and bioavailable form [19]. Among

MONPs, copper oxide nanoparticles (CuO NPs) have attracted increasing attention because copper is an essential micronutrient involved in photosynthetic electron transport (plastocyanin), respiration (cytochrome c oxidase), lignification, and antioxidant defense systems [30]. At optimal concentrations, CuO NPs have been shown to enhance germination efficiency, seedling vigor, antioxidant enzyme activity, and early growth in several crops, including *Brassica juncea*, maize, tomato, and rice [12, 18, 25, 38, 44]. These benefits are often linked to controlled Cu<sup>2+</sup> release, improved mobilization of seed reserves, and regulated reactive oxygen species (ROS) signaling, which collectively support rapid and uniform seed-to-seedling transition [18, 27, 34]. Despite growing evidence supporting CuO NP-based nano-priming in field and horticultural crops, systematic evaluation of this approach in okra remains limited. Existing nanoparticle research in okra has largely focused on foliar or soil application of MONPs such as SiO<sub>2</sub>, TiO<sub>2</sub>, and Al<sub>2</sub>O<sub>3</sub> under drought or salinity stress, demonstrating improved germination, photosynthetic performance, and stress tolerance [24, 42]. However, these studies do not directly address seed-based delivery of nanoparticles, nor do they integrate germination responses with downstream vegetative growth and yield attributes.

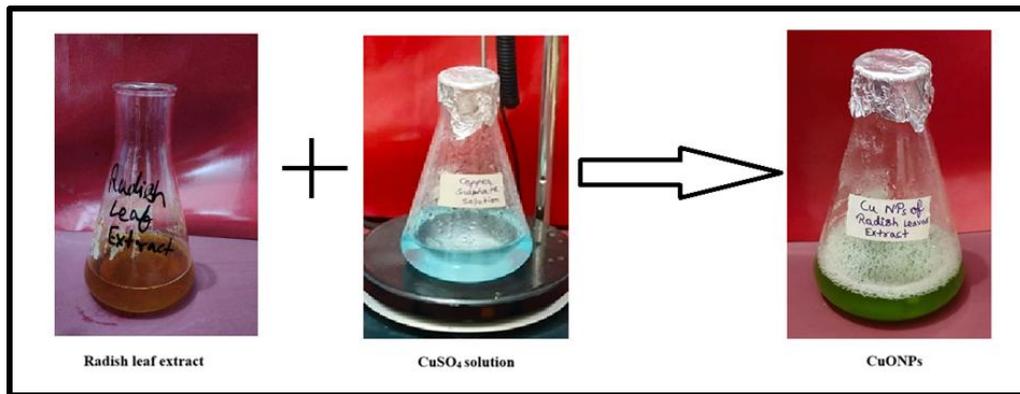
An additional limitation of many nanoparticle studies is the reliance on chemically synthesized nanomaterials, which raise concerns regarding environmental persistence and ecological safety. In contrast, green synthesis of nanoparticles using plant extracts offers a sustainable alternative, producing phytochemically capped nanoparticles with enhanced biocompatibility, reduced toxicity, and improved interaction with biological systems [8, 9, 28, 29]. The integration of green-synthesized CuO NPs into seed priming frameworks therefore represents a promising yet underexplored strategy for sustainable crop establishment. Accordingly, a critical research gap exists in understanding whether green-synthesized CuO nanoparticle-based seed priming can enhance germination, seedling vigor, vegetative growth, and yield of *A. esculentus* in a unified, stage-wise manner. Addressing this gap is particularly important given okra's sensitivity to early establishment and the increasing demand for eco-safe nano-enabled agricultural technologies [19, 45].

The novelty of the present study resides in the evaluation of green-synthesized CuO NPs as a seed-priming agent in okra (*A. esculentus*), a crop for which systematic nano-priming assessments remain limited. This work uniquely integrates a plant-mediated synthesis route using radish leaf extract with a seed-priming strategy, thereby coupling sustainability with functional nanotechnology. Unlike studies focusing solely on germination or early seedling responses, this investigation provides a comprehensive assessment linking germination behavior with subsequent seedling vigor, vegetative growth, pod traits, and final yield. By directly comparing CuO NP priming with ionic copper (CuSO<sub>4</sub>), radish extract, and conventional fertilizer treatments, the study elucidates the superior efficacy of nanoparticle-based copper delivery under standard growth conditions. The findings advance the concept of nano-priming as an eco-friendly and practical approach for improving crop establishment and productivity, extending its application to okra, an economically important horticultural crop.

## 2. Materials and Method

### 2.1. Green synthesis of CuO NPs

CuO NPs were synthesized using a green synthesis approach employing radish (*Raphanus sativus* L.) leaf extract as a reducing and stabilizing agent. Fresh radish leaves were thoroughly washed with distilled water, shade-dried, and finely powdered. The aqueous leaf extract was prepared by boiling the powdered material in distilled water, followed by filtration to remove insoluble residues. A CuSO<sub>4</sub> solution was prepared separately and heated at 30 ± 2 °C for 10 min under continuous stirring. The plant extract was then added dropwise to the copper salt solution with continuous stirring. The reaction mixture was kept undisturbed for 2 h, during which a visible color change indicated nanoparticle formation. The mixture was centrifuged for 10 min, and the resulting pellets were washed repeatedly with deionized water to remove unbound phytochemicals and dried overnight in a hot air oven to obtain CuO NP powder (*Figure 1*).



**Figure 1. Schematic Representation of the Green Synthesis of CuO NPs using Radish (*Raphanus Sativus* L.) Leaf Extract**

## 2.2. Characterization of CuO NP

The green-synthesized CuO NPs were characterized to confirm nanoparticle formation, crystallinity, morphology, elemental composition, and surface functional groups. Functional groups associated with phytochemical capping and Cu–O lattice formation were analyzed using Fourier Transform Infrared Spectroscopy (FTIR). Spectra were recorded over the range of 400–4000  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ , consistent with the reference methodology [10]. Surface morphology and particle distribution were examined using Field Emission Scanning Electron Microscopy (FESEM). Imaging was performed at accelerating voltages of 5–15 kV. Elemental composition and purity were confirmed using Energy Dispersive X-ray (EDX) spectroscopy, attached to the FESEM system. Nanoparticle surface charge and dispersion stability were assessed using zeta potential analysis with a dynamic light scattering analyzer. CuO NPs were dispersed in deionized water at a concentration of 0.1  $\text{mg mL}^{-1}$ , ultrasonicated for 15 min, and analyzed using folded capillary cells.

## 2.3. Experimental design and treatments

The study was conducted to evaluate the effects of different copper sources and concentrations on seed germination, seedling growth, vegetative development, reproductive traits, and yield under controlled and pot conditions. The experiment was laid out in a completely randomized design (CRD) comprising 13 treatments, including distilled water as a control, CuO NPs,  $\text{CuSO}_4$  solution, radish leaf extract, and nanofertilizer, each applied at 25, 50, and 100 ppm. Each treatment consisted of three independent biological replicates. All treatment solutions were freshly prepared using distilled water to achieve the desired ppm concentrations and applied uniformly throughout the experimental period, while the control received only distilled water.

## 2.4. Germination and seedling growth experiment

A seedling experiment was conducted to assess the effect of different treatments on seed germination and early seedling growth. Healthy and uniform seeds were surface sterilized using a Tween 20 (HI media, India) solution, followed by thorough rinsing with sterile distilled water to remove residual chemicals. Treated and control seeds were placed in sterile petri plates under controlled laboratory conditions. Germination was monitored daily, and germination percentage (%) was calculated based on the number of seeds showing visible radicle emergence relative to the total number of seeds sown. At the seedling stage, randomly selected seedlings from each treatment were harvested for growth analysis. The following parameters were recorded: Shoot length (cm), measured from the cotyledonary node to the shoot apex; root length (cm), measured from the cotyledonary node to the root tip; and total seedling length (cm), calculated as the sum of shoot and root length. Seedling fresh weight (g), recorded immediately after harvesting using

an analytical balance, and seedling dry weight (g), determined after oven-drying the seedlings at 60 °C until constant weight.

### 2.5. Pot experiment

A pot experiment was conducted to evaluate the effects of treatments on vegetative growth, reproductive traits, and yield up to the fruiting stage. Treated seeds were sown in pots under uniform growth conditions. Plant height was measured at 15, 30, and 45 days after germination (DAG) by recording the distance from the soil surface to the shoot apex using a graduated scale. These stages represented early seedling establishment (15 DAG), active vegetative growth (30 DAG), and advanced vegetative–reproductive transition (45 DAG). At maturity, pod number per plant was determined by counting all harvested pods. Pod length (cm) was measured using a vernier caliper from representative pods. Total yield per plant (g) was calculated by weighing all harvested pods using an analytical balance.

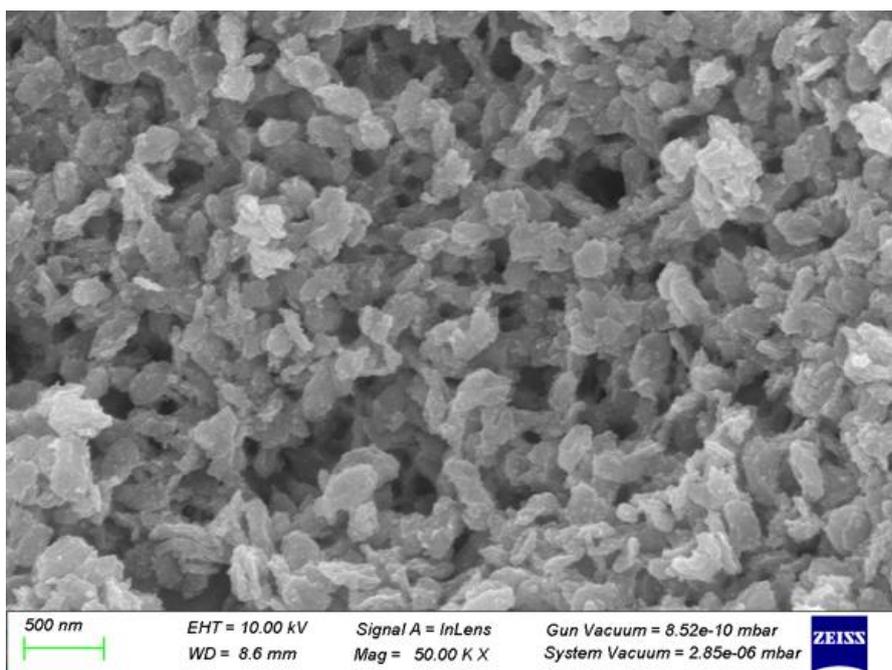
### 2.6. Statistical analysis

Prior to inferential analysis, data were tested for compliance with parametric assumptions. Normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene’s test. Seedling-stage data, which did not consistently meet normality assumptions, were analyzed using the Kruskal–Wallis non-parametric test. When significant differences were detected ( $p < 0.05$ ), Dunn’s post hoc test with Bonferroni correction was applied. Relationships among germination percentage, seedling growth parameters, and biomass traits were examined using Spearman’s rank correlation coefficients. All pot experiment variables satisfied assumptions of normality ( $p > 0.05$ ) and variance homogeneity ( $p > 0.05$ ), validating the use of parametric tests. Pot experiment data were analyzed using one-way analysis of variance (ANOVA). When the ANOVA F-test indicated significant treatment effects ( $p < 0.05$ ), Duncan’s Multiple Range Test (DMRT) was applied for pairwise comparison of treatment means. Associations among vegetative growth stages, pod traits, and total yield were examined using Pearson’s correlation coefficient ( $r$ ). All statistical analyses were performed using IBM SPSS Statistics software (version 27; IBM Corp., Armonk, NY, USA), and graphical outputs were prepared using OriginPro software (version 8.5; OriginLab Corporation, Northampton, MA, USA).

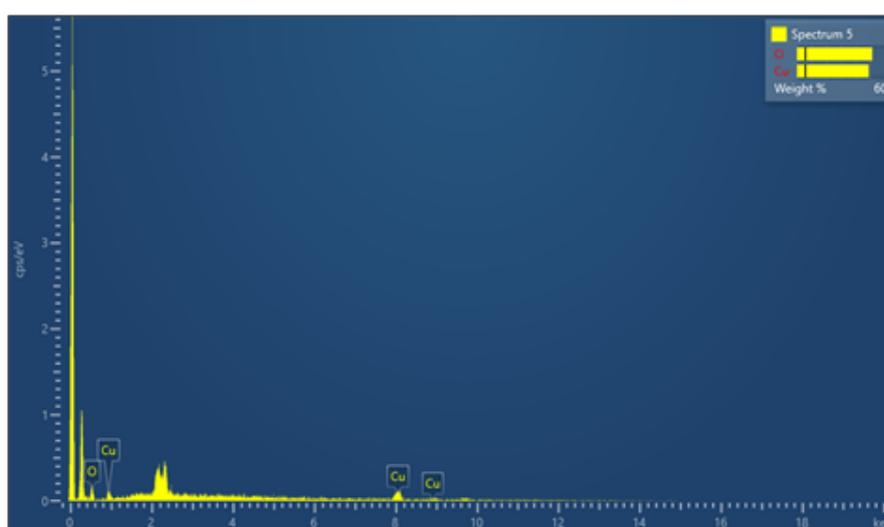
## 3. Results

### 3.1. Characterization of CuO NP

The synthesized CuO NPs predominantly exhibited a near-spherical morphology with particle sizes ranging from approximately 150–250 nm, along with the presence of some agglomerated clusters. The relatively uniform size distribution and consistent particle shape indicate successful nanoparticle formation under controlled synthesis conditions (*Figure 2*). Energy-dispersive X-ray spectroscopy (EDS) analysis confirmed the elemental composition of the CuO NPs, revealing 48.80 wt% copper (Cu) and 51.20 wt% oxygen (O), with corresponding atomic percentages of 19.35% Cu and 80.65% O, thereby validating the formation of CuO NPs (*Figure 3*).

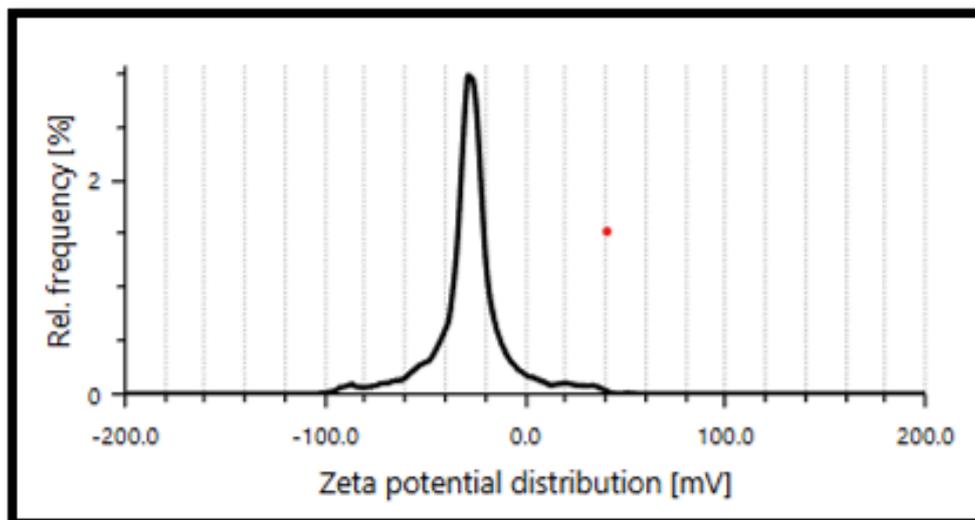


**Figure 2. FESEM Micrograph of Green-Synthesized CuO NPs**



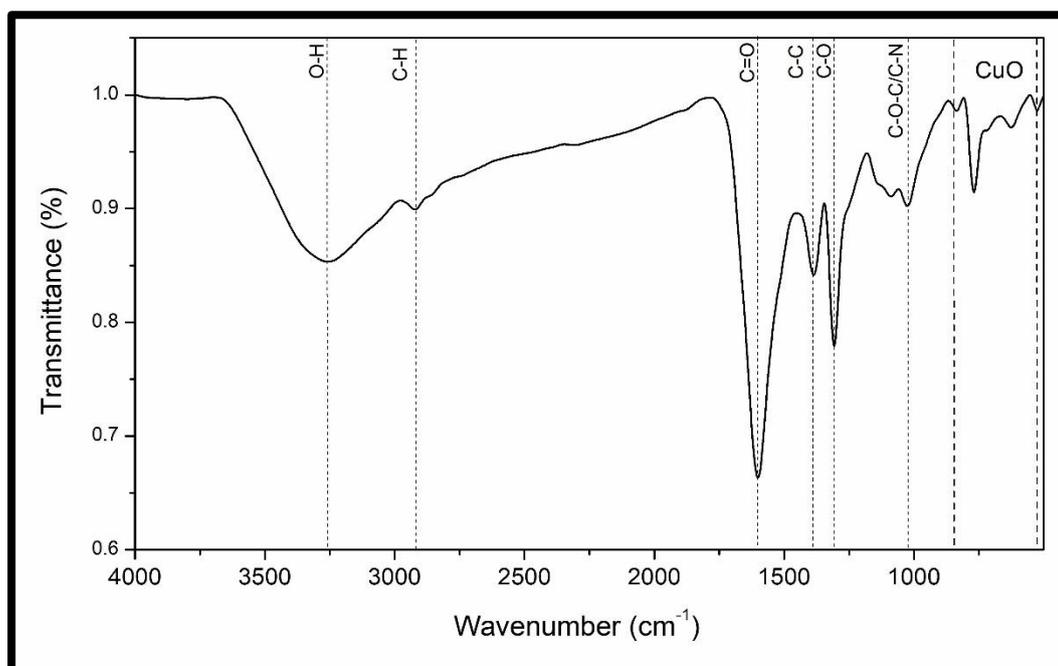
**Figure 3. Energy-Dispersive X-Ray Spectroscopy (EDS) Spectrum of Green-Synthesized CuO NPs**

Zeta potential analysis showed a dominant peak at  $-23.21$  mV, with a relative frequency of 2.23%, suggesting moderate colloidal stability of the nanoparticle suspension. The zeta potential distribution spanned a range from approximately  $-60$  to  $+20$  mV, with minor secondary shoulders observed near  $-100$  mV and  $+60$  mV, indicating the presence of heterogeneous surface charge populations within the CuO NPs dispersion (*Figure 4*).



**Figure 4. Zeta Potential Distribution of Green-Synthesized CuO NPs**

The FTIR spectrum of the synthesized CuO NPs displayed distinct absorption bands corresponding to both surface functional groups and metal–oxygen vibrations (Figure X). A broad absorption band observed around  $\sim 3400\text{ cm}^{-1}$  is attributed to O–H stretching vibrations, indicating the presence of surface hydroxyl groups or adsorbed moisture. A weak band near  $\sim 2920\text{--}3000\text{ cm}^{-1}$  corresponds to C–H stretching vibrations, suggesting the presence of residual organic moieties associated with the synthesis process. A strong and sharp absorption band centered at  $\sim 1630\text{--}1650\text{ cm}^{-1}$  was assigned to C=O stretching vibrations, while additional bands in the region of  $\sim 1300\text{--}1400\text{ cm}^{-1}$  correspond to C–C and C–O stretching vibrations. The absorption observed around  $\sim 1000\text{--}1100\text{ cm}^{-1}$  is attributed to C–O–C and C–N vibrations, indicating surface-bound functional groups. Importantly, a characteristic absorption band appearing in the low wavenumber region below  $\sim 800\text{ cm}^{-1}$  corresponds to the Cu–O stretching vibration, confirming the successful formation of CuO NPs (Figure 5).



**Figure 5. Fourier Transform Infrared (FTIR) Spectrum of Green-Synthesized CuO NPs**

### 3.2. Effect on Germination and Seedling parameters

Descriptive statistics for seedling traits revealed substantial variability across parameters. Germination showed a median of 100% with an IQR of 16.7, indicating high germination capacity but with noticeable dispersion among observations. The Shapiro–Wilk test confirmed non-normal distribution for germination ( $W = 0.68, p < 0.001$ ). Among growth traits, the median shoot length was 1.76 cm (IQR = 0.75) and root length was 0.87 cm (IQR = 0.50). Shoot length data conformed to normal distribution ( $W = 0.974, p = 0.196$ ), whereas root length deviated significantly from normality ( $W = 0.944, p = 0.005$ ). The seedling length, with a median of 2.66 cm and IQR of 1.0, exhibited normal distribution ( $W = 0.981, p = 0.432$ ). Biomass-related traits showed comparatively lower central values. Fresh weight (FW) recorded a median of 0.80 g (IQR = 0.25), while dry weight (DW) had a median of 0.37 g (IQR = 0.14). Both FW ( $W = 0.875, p < 0.001$ ) and DW ( $W = 0.917, p < 0.001$ ) significantly deviated from normality, indicating skewed distributions. The Shapiro–Wilk analysis demonstrated that while shoot length and seedling length followed normal distribution, germination percentage, root length, and biomass traits (FW and DW) were non-normally distributed, justifying the use of non-parametric statistical tests for subsequent analyses of these parameters.

The Kruskal–Wallis test was applied to evaluate differences among treatments for germination and seedling growth traits. Germination percentage did not differ significantly among treatments ( $\chi^2 = 14.7, p = 0.260$ ), with a moderate effect size ( $\epsilon^2 = 0.229$ ). Similarly, shoot length showed no significant treatment effect ( $\chi^2 = 12.2, p = 0.429; \epsilon^2 = 0.191$ ). In contrast, root length differed significantly among treatments ( $\chi^2 = 23.2, p = 0.026$ ), exhibiting a moderate-to-large effect size ( $\epsilon^2 = 0.363$ ). Seedling length did not show a statistically significant difference ( $\chi^2 = 17.1, p = 0.144$ ), although the effect size was moderate ( $\epsilon^2 = 0.268$ ), indicating appreciable biological variation despite statistical non-significance. Biomass traits responded differentially to treatments. Fresh weight (FW) showed a highly significant difference among treatments ( $\chi^2 = 29.1, p = 0.004$ ) with a large effect size ( $\epsilon^2 = 0.454$ ), highlighting strong treatment influence on seedling biomass accumulation. Conversely, dry weight (DW) did not vary significantly ( $\chi^2 = 13.2, p = 0.354$ ), with a moderate effect size ( $\epsilon^2 = 0.206$ ). The Kruskal–Wallis analysis indicates that root growth and fresh biomass are the most responsive traits, showing significant treatment-dependent variation, whereas germination, shoot length, seedling length, and dry weight remained statistically comparable across treatments.

Treatment-wise medians and interquartile ranges (IQRs) revealed clear differences in germination, growth, and biomass traits across copper sources applied at different concentrations (Table 1).

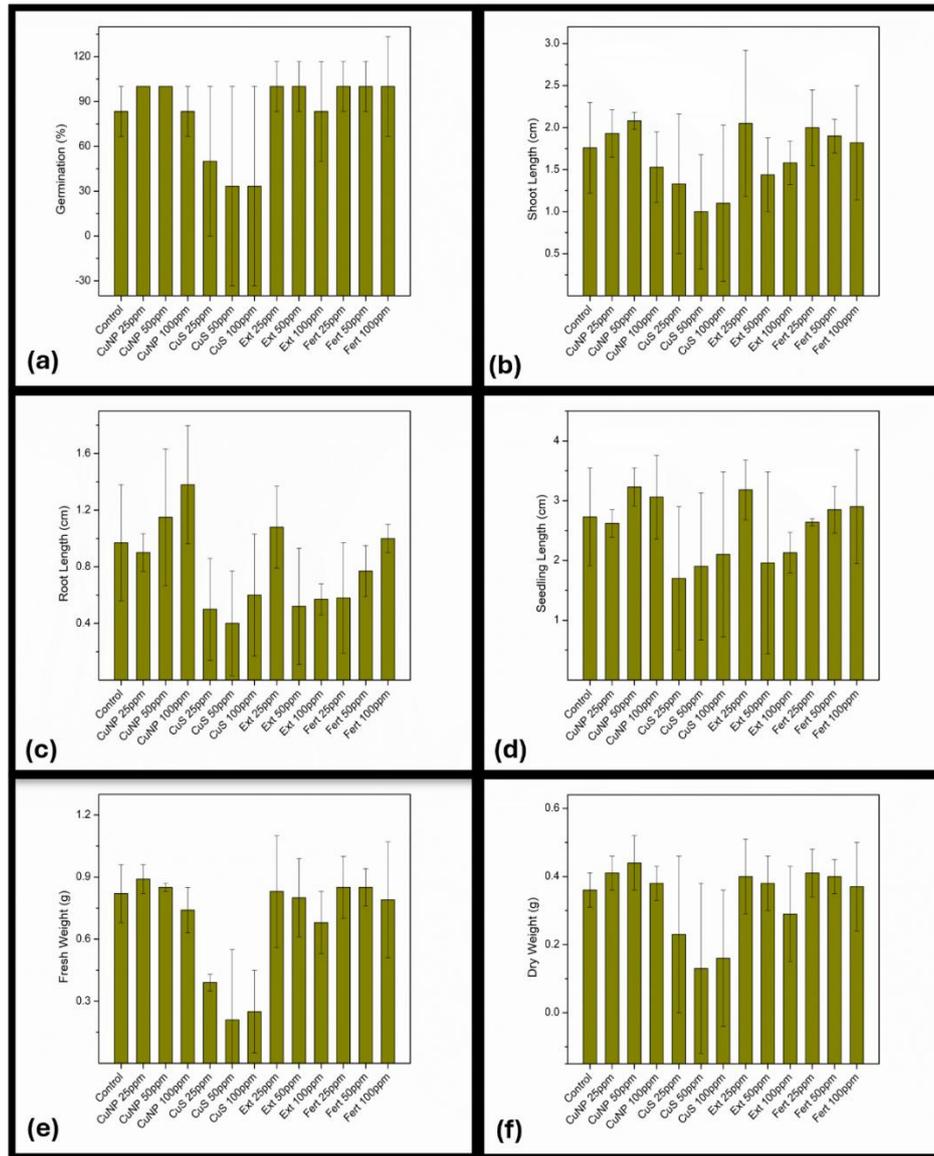
**Table 1. Effect of Different Seed-Priming Treatments on Germination Efficiency and Seedling Growth Traits of Okra (*A. esculentus* L.) under Laboratory Conditions**

Treatment	Germination Efficiency (%)	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)	Fresh Weight (g)	Dry Weight (g)
Distilled water	83.3±16.7	1.8±0.5	1.0±0.4	2.7±0.1	0.8±0.1	0.4±0.1
CuO NPs 25 ppm	100.0±0.0	1.9±0.3	0.9±0.1	2.6±0.2	0.9±0.1	0.4±0.1
CuO NPs 50 ppm	100.0±0.0	2.1±0.1	1.2±0.5	3.2±0.3	0.9±0.0	0.4±0.1
CuO NPs 100 ppm	83.3±16.7	1.5±0.4	1.4±0.4	3.1±0.7	0.7±0.1	0.4±0.1
CuSO <sub>4</sub> 25 ppm	50.0±50.0	1.3±0.8	0.5±0.4	1.7±1.2	0.4±0.0	0.2±0.2
CuSO <sub>4</sub> 50 ppm	33.3±83.0	1.0±0.7	0.4±0.4	1.9±1.2	0.2±0.3	0.1±0.3

<b>CuSO<sub>4</sub> 100 ppm</b>	33.3±66.7	1.1±0.9	0.6±0.4	2.1±1.4	0.3±0.2	0.2±0.2
<b>Radish Extract 25 ppm</b>	100.0±16.7	2.1±0.9	1.1±0.3	3.2±0.5	0.8±0.3	0.4±0.1
<b>Radish Extract 50 ppm</b>	100.0±16.7	1.4±0.4	0.5±0.4	2.0±1.5	0.8±0.2	0.4±0.1
<b>Radish Extract 100 ppm</b>	83.3±33.3	1.6±0.3	0.6±0.1	2.1±0.3	0.7±0.2	0.3±0.1
<b>Fertilizer 25 ppm</b>	100.0±16.7	2.0±0.5	0.6±0.4	2.6±0.1	0.9±0.2	0.4±0.1
<b>Fertilizer 50 ppm</b>	100.0±16.7	1.9±0.2	0.8±0.2	2.9±0.4	0.9±0.1	0.4±0.1
<b>Fertilizer 100 ppm</b>	100.0±33.3	1.8±0.7	1.0±0.1	2.9±1.0	0.8±0.3	0.4±0.1

*Values are presented as mean ± standard deviation (SD)*

Germination percentage reached the maximum median value (100%) under CuO NPs at 25 and 50 ppm, radish leaf extract at 25 and 50 ppm, and all fertilizer concentrations, indicating strong stimulation of seed germination at these ppm levels. In contrast, CuSO<sub>4</sub> solution showed a pronounced inhibitory effect, with median germination declining to 50% at 25 ppm and 33.3% at 50 and 100 ppm, accompanied by large IQR values (50–83.3), reflecting high variability and stress-induced inhibition. The control (distilled water) showed moderate germination (83.3%; IQR = 16.7). For shoot length, the highest median values were recorded under CuO NPs at 50 ppm (2.08 cm), radish leaf extract at 25 ppm (2.05 cm), and fertilizer at 25 ppm (2.00 cm), whereas CuSO<sub>4</sub> treatments consistently resulted in reduced shoot growth (1.0–1.33 cm). Variability in shoot length was also higher under CuSO<sub>4</sub>, as indicated by wider IQRs. Root length showed strong sensitivity to copper source and concentration, with maximum median values observed under CuO NPs at 100 ppm (1.38 cm) and 50 ppm (1.15 cm). In contrast, CuSO<sub>4</sub> treatments suppressed root elongation (0.4–0.6 cm), indicating ionic Cu toxicity at comparable ppm levels. Consequently, seedling length was greatest under CuO NPs at 50 ppm (3.23 cm) and radish leaf extract at 25 ppm (3.18 cm), followed by CuO NPs at 100 ppm (3.06 cm). Seedlings exposed to CuSO<sub>4</sub> exhibited the lowest median lengths (1.7–2.1 cm) with high IQRs (1.2–1.38), indicating inconsistent and inhibitory growth responses. Biomass accumulation followed similar trends. Fresh weight (FW) was highest under CuO NPs at 25 ppm (0.89 g) and under CuO NPs at 50 ppm and fertilizer treatments (0.85 g), whereas CuSO<sub>4</sub> treatments caused sharp reductions in FW (0.21–0.39 g). Dry weight (DW) peaked under CuO NPs at 50 ppm (0.44 g) and remained relatively high under fertilizer and radish leaf extract treatments (0.38–0.41 g) but declined markedly under CuSO<sub>4</sub>, particularly at 50 ppm (0.13 g). The median–IQR analysis demonstrates that CuO NPs (especially 25–50 ppm) and radish leaf extract at lower ppm levels enhance seedling vigor and biomass, whereas CuSO<sub>4</sub> at equivalent ppm concentrations exerts phytotoxic effects, leading to reduced germination, growth, and biomass with greater variability (*Figure 6*).



**Figure 6. Effect of Different Copper Sources and Concentrations on Germination and Seedling Growth Traits of Okra (*A. esculentus* L.). (a) Germination Percentage, (b) Shoot Length, (c) Root Length, (d) Seedling Length, (e) Fresh Weight, And (f) Dry Weight under Control and Treatment Conditions. Bars Represent Mean Values with Error Bars Indicating Variability Among Replicates.**

### 3.3. Correlation Analysis among Germination, Growth, and Biomass Traits

Non-parametric correlation analysis using Spearman’s rank correlation ( $\rho$ ) and Kendall’s Tau-b ( $\tau$ ) revealed strong and consistent relationships among germination, seedling growth, and biomass traits (*Table 2*).

**Table 2. Non-Parametric Correlation Analysis among Germination, Seedling Growth, and Biomass Traits of Okra (*A. esculentus* L.) under Different Seed-Priming Treatments**

Correlation	Germination Efficiency	Shoot Length	Root Length	Seedling Length	Fresh Weight	Dry Weight

<b>Germination Efficiency</b>	Spearman's rho	—					
	p-value	—					
	Kendall's Tau B	—					
	p-value	—					
<b>Shoot Length</b>	Spearman's rho	0.599***	—				
	p-value	<.001	—				
	Kendall's Tau B	0.486***	—				
	p-value	<.001	—				
<b>Root Length</b>	Spearman's rho	0.327**	0.234	—			
	p-value	0.008	0.061	—			
	Kendall's Tau B	0.244*	0.164	—			
	p-value	0.011	0.054	—			
<b>Seedling Length</b>	Spearman's rho	0.519***	0.723** *	0.786** *	—		
	p-value	<.001	<.001	<.001	—		
	Kendall's Tau B	0.406***	0.568** *	0.587** *	—		
	p-value	<.001	<.001	<.001	—		
<b>Fresh Weight</b>	Spearman's rho	0.746***	0.617** *	0.414** *	0.648** *	—	
	p-value	<.001	<.001	<.001	<.001	—	
	Kendall's Tau B	0.651***	0.459** *	0.282** *	0.467** *	—	
	p-value	<.001	<.001	<.001	<.001	—	
<b>Dry Weight</b>	Spearman's rho	0.708***	0.460** *	0.416** *	0.522** *	0.676** *	—
	p-value	<.001	<.001	<.001	<.001	<.001	—
	Kendall's Tau B	0.616***	0.324** *	0.299** *	0.370** *	0.524** *	—
	p-value	<.001	<.001	<.001	<.001	<.001	—

Significance levels are indicated as  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*).

Germination percentage showed significant positive correlations with all growth and biomass parameters. The strongest associations were observed with fresh weight ( $\rho = 0.746$ ,  $\tau = 0.651$ ;  $p < 0.001$ ) and dry weight ( $\rho = 0.708$ ,  $\tau = 0.616$ ;  $p < 0.001$ ), indicating that higher germination was closely linked to improved biomass accumulation. Germination was also moderately to strongly correlated with shoot length ( $\rho = 0.599$ ;  $p < 0.001$ ) and seedling length ( $\rho = 0.519$ ;  $p < 0.001$ ), while its association with root length was weaker but still significant ( $\rho = 0.327$ ;  $p = 0.008$ ). Among vegetative traits, shoot length and root length showed a weak and statistically non-significant relationship ( $\rho = 0.234$ ;  $p = 0.061$ ), suggesting partially independent growth responses. In contrast, seedling length exhibited very strong positive correlations with both shoot length ( $\rho = 0.723$ ;  $p < 0.001$ ) and root length ( $\rho = 0.786$ ;  $p < 0.001$ ), confirming that overall seedling size was jointly determined by both shoot and root development. Biomass traits were closely associated with growth parameters. Fresh weight (FW) showed strong positive correlations with seedling length ( $\rho = 0.648$ ;  $p < 0.001$ ) and shoot length ( $\rho = 0.617$ ;  $p < 0.001$ ), and a moderate correlation with root length ( $\rho = 0.414$ ;  $p < 0.001$ ). Similarly, dry weight (DW)

correlated significantly with fresh weight ( $\rho = 0.676$ ;  $p < 0.001$ ), reflecting coordinated biomass accumulation, and also showed moderate associations with seedling length ( $\rho = 0.522$ ;  $p < 0.001$ ), shoot length ( $\rho = 0.460$ ;  $p < 0.001$ ), and root length ( $\rho = 0.416$ ;  $p < 0.001$ ). The concordant results of Spearman’s  $\rho$  and Kendall’s  $\tau$  analyses demonstrate that enhanced germination is strongly linked to improved seedling growth and biomass, with seedling length acting as a central integrative trait connecting shoot, root, and biomass responses under different copper treatments.

### 1.1. Effect on Growth and Fruit Yield

The mean performance, variability, and normality of growth and yield traits are as follows; Mean plant height increased with crop age, recording  $8.09 \pm 1.45$  cm at 15 DAG,  $30.8 \pm 5.79$  cm at 30 DAG, and  $55.5 \pm 12.9$  cm at 45 DAG, indicating progressive vegetative development with increasing variability at later stages. Reproductive traits showed mean values of  $5.59 \pm 1.65$  pods plant<sup>-1</sup> and  $9.42 \pm 1.88$  cm pod length, while total yield per plant averaged  $33.2 \pm 15.8$  g, reflecting substantial dispersion among treatments. Normality was assessed using the Shapiro–Wilk test, which indicated that all measured traits conformed to a normal distribution ( $p > 0.05$ ). Specifically, Shapiro–Wilk p-values ranged from 0.768 to 0.932 for growth traits and pod length, while pod number ( $p = 0.102$ ) and total yield ( $p = 0.070$ ) also satisfied the normality assumption, albeit marginally in the case of yield. The results confirm that all variables met the assumptions of normality, supporting the application of parametric statistical analyses (one-way ANOVA) for evaluating treatment effects on growth, pod traits, and yield. Combined with the confirmed homogeneity of variances (Levene’s test,  $p > 0.05$ ), these findings justify the use of ANOVA followed by Tukey’s HSD post hoc test for multiple comparisons.

One-way analysis of variance (ANOVA) revealed significant differences among treatments for all measured growth, reproductive, and yield parameters. Plant height varied significantly with treatment at all growth stages. At 15 DAG, treatment effects were significant ( $F_{12,26} = 2.90$ ,  $p = 0.011$ ). The magnitude of treatment effects increased at later stages, with highly significant differences observed at 30 DAG ( $F_{12,26} = 7.17$ ,  $p < 0.001$ ) and 45 DAG ( $F_{12,26} = 11.40$ ,  $p < 0.001$ ), indicating progressive divergence in vegetative growth among treatments. Reproductive traits were also strongly influenced by treatment. Pod number differed significantly among treatments ( $F_{12,26} = 10.28$ ,  $p < 0.001$ ), demonstrating variable reproductive performance. Similarly, pod length showed a highly significant treatment effect ( $F_{12,26} = 6.51$ ,  $p < 0.001$ ), reflecting differential pod development. The most pronounced treatment effect was observed for total yield per plant, which exhibited a very high F value ( $F_{12,26} = 55.87$ ,  $p < 0.001$ ), indicating that treatment application had a substantial impact on final productivity. The ANOVA results confirm that treatments significantly affected vegetative growth across all stages, pod characteristics, and yield, with the strongest responses evident for yield and later growth stages.

Treatment-wise mean values ( $\pm$  SD) showed pronounced differences in vegetative growth, reproductive traits, and yield among treatments applied at different ppm concentrations (Table 3).

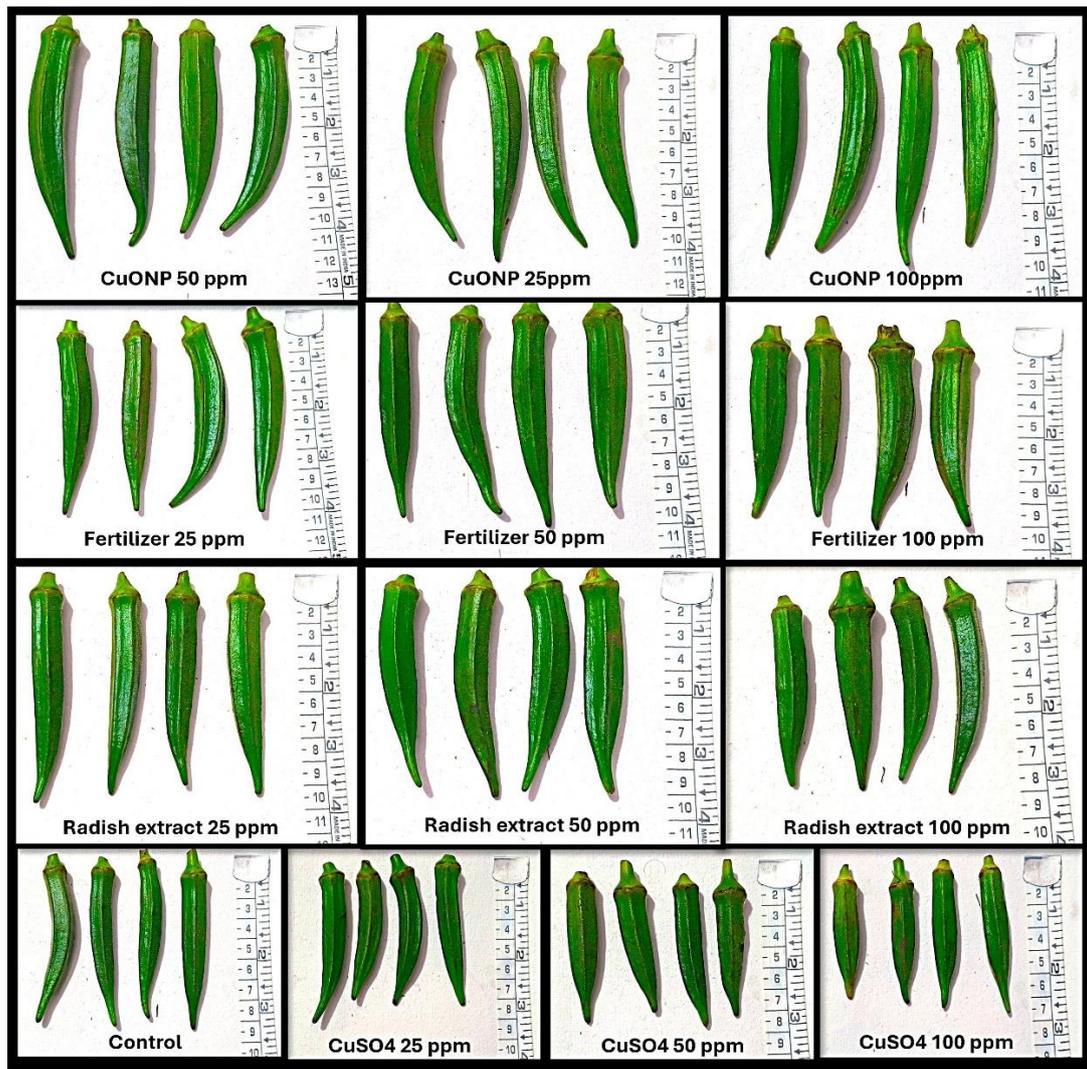
**Table 3. Effect of Different Seed-Priming Treatments on Vegetative Growth, Pod Traits, and Yield of Okra (*A. esculentus* L.) under Pot Conditions**

Treatment	15 DAG (cm)	30 DAG (cm)	45 DAG (cm)	Pod Number	Pod Length (cm)	Total Yield (g)
Distilled Water	7.4±0.8	27.0±1.5	52.0±6.3	5.0±0.0	8.2±0.2	21.9±1.1
CuO NPs 25 ppm	9.1±2.0	36.2±2.3	69.0±10.5	7.0±1.0	11.8±1.2	53.4±6.8
CuO NPs 50 ppm	9.8±1.5	39.0±4.0	72.0±7.5	8.0±1.0	11.6±1.5	61.1±3.4

<b>CuO NPs 100 ppm</b>	7.9±0.5	35.1±4.0	58.0±5.3	6.0±1.0	10.2±0.8	48.6±6.4
<b>CuSO<sub>4</sub> 25 ppm</b>	6.8±0.5	24.8±2.5	38.0±5.0	4.0±1.0	7.9±1.0	16.4±2.4
<b>CuSO<sub>4</sub> 50 ppm</b>	6.8±1.1	23.4±2.5	36.0±5.0	3.3±0.6	7.6±1.0	12.0±1.4
<b>CuSO<sub>4</sub> 100 ppm</b>	6.3±2.3	22.0±6.8	35.0±11.0	2.7±0.6	6.0±0.8	12.5±1.9
<b>Radish Extract 25 ppm</b>	8.6±1.1	32.2±4.8	60.8±3.8	6.0±1.0	9.9±1.1	33.9±3.3
<b>Radish Extract 50 ppm</b>	8.7±0.5	30.4±3.6	56.0±5.0	6.0±0.0	9.9±1.0	32.8±1.3
<b>Radish Extract 100 ppm</b>	7.1±0.5	29.1±3.0	56.5±2.5	5.0±1.0	9.0±1.8	19.6±3.1
<b>Fertilizer 25 ppm</b>	8.6±0.5	34.0±3.0	64.5±5.0	7.0±1.0	9.2±0.9	43.3±5.6
<b>Fertilizer 50 ppm</b>	8.6±0.5	33.8±0.5	63.5±5.0	6.7±0.6	11.0±1.2	40.5±1.9
<b>Fertilizer 100 ppm</b>	9.5±1.0	33.2±2.5	60.0±2.5	6.0±1.0	10.1±1.5	35.2±3.2

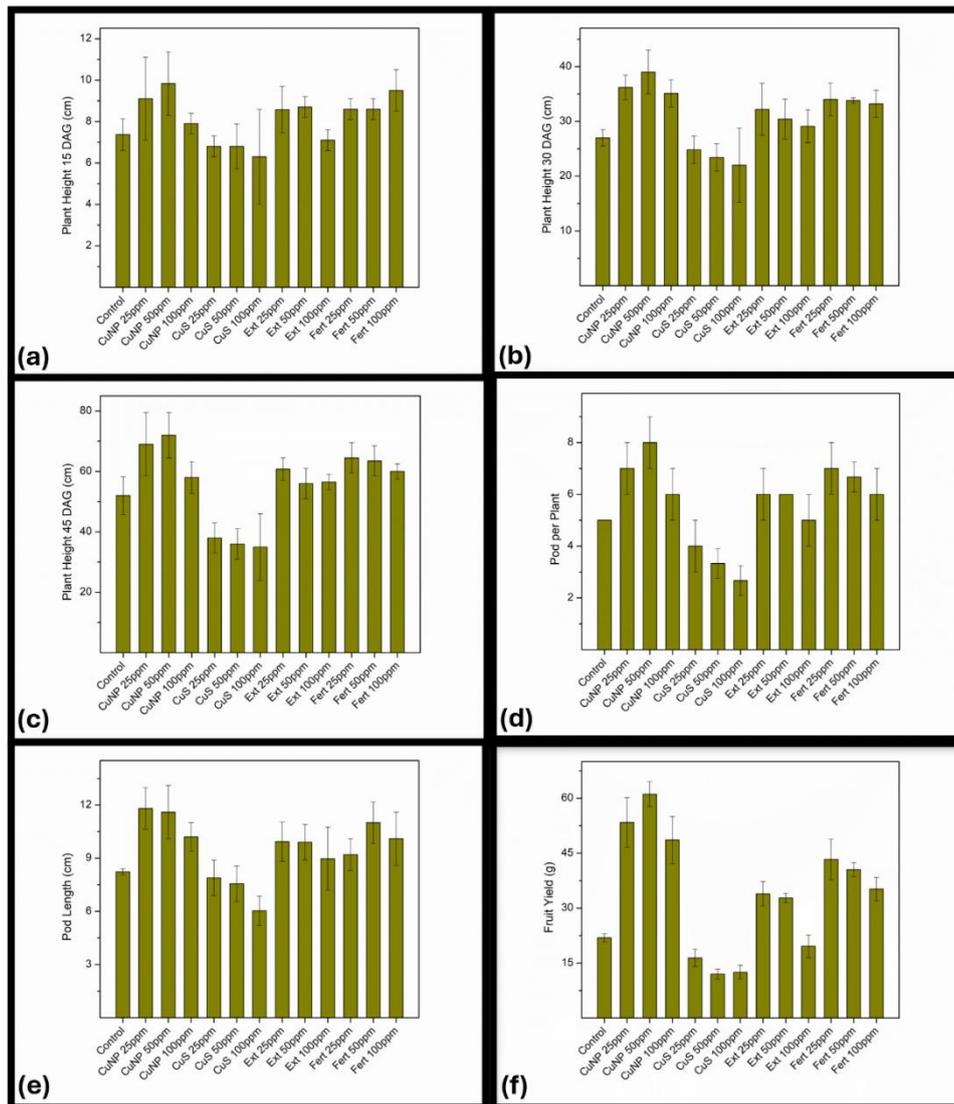
Plant height increased progressively from 15 to 45 DAG across all treatments, with clear variation depending on copper source and concentration. At 15 DAG, the highest mean plant height was observed under CuO NPs at 50 ppm ( $9.83 \pm 1.53$  cm) and fertilizer at 100 ppm ( $9.50 \pm 1.00$  cm), followed by CuO NPs at 25 ppm ( $9.10 \pm 2.00$  cm). In contrast, CuSO<sub>4</sub> solution, particularly at 100 ppm ( $6.30 \pm 2.29$  cm), resulted in reduced early growth. Similar trends were evident at 30 DAG, where CuO NPs at 50 ppm ( $39.0 \pm 4.0$  cm) and CuO NPs at 25 ppm ( $36.2 \pm 2.25$  cm) produced the tallest plants, whereas CuSO<sub>4</sub> treatments recorded consistently lower heights (22.0–24.8 cm). By 45 DAG, maximum plant height was recorded under CuO NPs at 50 ppm ( $72.0 \pm 7.5$  cm), followed by CuO NPs at 25 ppm ( $69.0 \pm 10.5$  cm) and fertilizer at 25–50 ppm (63.5–64.5 cm). The lowest plant height was observed under CuSO<sub>4</sub> at 100 ppm ( $35.0 \pm 11.0$  cm), indicating strong growth inhibition at higher ionic Cu levels.

Reproductive traits responded similarly. Pod number was highest under CuO NPs at 50 ppm ( $8.0 \pm 1.0$  pods plant<sup>-1</sup>) and under CuO NPs at 25 ppm and fertilizer at 25 ppm ( $7.0 \pm 1.0$  pods). Increasing concentrations of CuSO<sub>4</sub> caused a progressive reduction in pod number, reaching a minimum at 100 ppm ( $2.67 \pm 0.58$  pods plant<sup>-1</sup>). Pod length was greatest under CuO NPs at 25 ppm ( $11.8 \pm 1.17$  cm) and 50 ppm ( $11.6 \pm 1.50$  cm), while the shortest pods were recorded under CuSO<sub>4</sub> at 100 ppm ( $6.03 \pm 0.83$  cm). These treatment effects were reflected in total yield per plant. The highest mean yield was obtained with CuO NPs at 50 ppm ( $61.1 \pm 3.4$  g), followed by CuO NPs at 25 ppm ( $53.4 \pm 6.8$  g) and CuO NPs at 100 ppm ( $48.6 \pm 6.4$  g). Fertilizer treatments resulted in moderate yields (35.2–43.3 g plant<sup>-1</sup>), while radish leaf extract at 25–50 ppm produced intermediate yields (32.8–33.9 g plant<sup>-1</sup>). In contrast, CuSO<sub>4</sub> solution markedly reduced yield, with the lowest values observed at 50–100 ppm (12.0–12.5 g plant<sup>-1</sup>) (Figures 7 and 8).



**Figure 7. Representative Images of Okra Pods Harvested from Plants Grown under Different Seed-Priming Treatments**

The mean  $\pm$  SD analysis confirms that CuO NPs, particularly at 25–50 ppm, significantly enhanced vegetative growth, pod development, and yield, whereas CuSO<sub>4</sub> at equivalent ppm concentrations exerted inhibitory effects. Radish leaf extract and fertilizer treatments supported moderate growth and productivity, highlighting the importance of copper form and concentration in determining plant performance under pot conditions.



**Figure 8. Effect of Different Seed-Priming Treatments on Vegetative Growth, Reproductive Traits, and Yield of Okra (*A. esculentus* L.). (a) Plant Height at 15 Days After Germination (DAG), (b) Plant Height at 30 DAG, (c) Plant Height at 45 DAG, (d) Number of Pods Per Plant, (e) Pod Length, and (f) Total Fruit Yield Per Plant Under Control and Various Copper-Based Treatments. Bars Represent Mean Values with Error Bars Indicating Standard Deviation.**

**1.2. Pearson’s Correlation Analysis of Growth, Pod Traits, and Yield**

Pearson’s correlation analysis revealed strong and highly significant positive relationships among vegetative growth stages, pod traits, and total yield (*Table 4*), indicating a close developmental linkage between early growth, reproductive performance, and final productivity.

**Table 4. Pearson’s Correlation Matrix Showing Relationships among Vegetative Growth Stages, Pod Traits, and Total Yield of Okra (*A. esculentus* L.) under Different Seed-Priming Treatments**

Correlation Matrix		15 DAG	30 DAG	45 DAG	Pod Number	Pod Length	Total Yield
15 DAG	Pearson's r	—					
	p-value	—					

30 DAG	Pearson's r	0.897** *	—				
	p-value	<.001	—				
45 DAG	Pearson's r	0.886** *	0.959** *	—			
	p-value	<.001	<.001	—			
Pod Number	Pearson's r	0.914** *	0.968** *	0.972** *	—		
	p-value	<.001	<.001	<.001	—		
Pod Length	Pearson's r	0.886** *	0.940** *	0.915** *	0.924***	—	
	p-value	<.001	<.001	<.001	<.001	—	
Total Yield	Pearson's r	0.860** *	0.970** *	0.898** *	0.932***	0.897***	—
	p-value	<.001	<.001	<.001	<.001	<.001	—

Significance levels are indicated as  $p < 0.001$  (\*\*\*)

Plant height at 15 DAG showed a strong positive correlation with height at 30 DAG ( $r = 0.897$ ,  $p < 0.001$ ) and 45 DAG ( $r = 0.886$ ,  $p < 0.001$ ), demonstrating consistent growth ranking among treatments from early to later stages. An even stronger association was observed between 30 and 45 DAG plant height ( $r = 0.959$ ,  $p < 0.001$ ), reflecting sustained vegetative development across growth phases. Vegetative growth was strongly associated with reproductive traits. Plant height at all stages correlated significantly with pod number ( $r = 0.914$ – $0.972$ ,  $p < 0.001$ ) and pod length ( $r = 0.886$ – $0.940$ ,  $p < 0.001$ ), indicating that enhanced early and mid-stage growth translated directly into improved pod formation and elongation. Total yield per plant exhibited strong positive correlations with plant height at 15 DAG ( $r = 0.860$ ), 30 DAG ( $r = 0.970$ ), and 45 DAG ( $r = 0.898$ ) ( $p < 0.001$  for all), highlighting the importance of vegetative vigor—particularly at 30 DAG—in determining final yield. Yield was most strongly correlated with pod number ( $r = 0.932$ ,  $p < 0.001$ ), followed by pod length ( $r = 0.897$ ,  $p < 0.001$ ), confirming that pod production was the principal driver of yield variation among treatments. The Pearson's correlation results demonstrate that early vegetative growth establishes a growth trajectory that strongly governs reproductive traits and final yield under different copper treatments. The consistently high correlation coefficients underscore the integrative role of vegetative vigor in maximizing productivity under pot conditions.

## 2. Discussion

The present study demonstrates that green-synthesized CuO NPs exert pronounced concentration-dependent effects on seedling vigor, vegetative growth, reproductive traits, and final yield of *A. esculentus*. In contrast, equivalent concentrations of ionic copper supplied as CuSO<sub>4</sub> resulted in growth inhibition and yield penalties. By integrating detailed nanoparticle characterization with multi-stage biological responses, the findings clearly indicate that CuO NP physicochemical properties—size, surface chemistry, and colloidal stability—are decisive determinants of plant performance, supporting current nano-biointeraction models for metal oxide nanonutrients [15, 16].

### 2.1. CuO NPs Properties and Their Biological Significance

The near-spherical morphology and nanoscale size range (150–250 nm), combined with moderate colloidal stability (zeta potential  $-23.21$  mV), suggest that the synthesized CuO NPs were well suited for biological interaction. Nanoparticles within this size domain can effectively interact with root epidermal surfaces and apoplastic pathways while limiting uncontrolled aggregation and excessive translocation [11, 28]. FTIR-confirmed Cu–O vibrations and surface

functional groups derived from radish leaf extract further imply phytochemical capping, which likely moderated  $\text{Cu}^{2+}$  release and enhanced biocompatibility [8, 26]. Such controlled release behavior distinguishes nanoparticulate copper from bulk salts and underpins the superior growth and yield responses observed.

## 2.2. Germination and Early Seedling Establishment

Germination percentage was only modestly affected by CuO NP treatments and did not differ significantly from the control, indicating that CuO NPs did not compromise seed viability. This observation is consistent with earlier studies showing that copper nanoparticles at low to moderate concentrations exert minimal effects on germination but strongly influence post-germinative growth [20, 30]. Enhanced early vigor at 25–50 ppm CuO NPs may be attributed to improved seed coat permeability, facilitated water imbibition, and rapid metabolic reactivation, as reported for other biogenic metal oxide nanoparticles [1, 22, 32, 39]. In contrast, the marked reduction in germination under  $\text{CuSO}_4$  treatments reflects ionic copper toxicity, which disrupts membrane integrity and induces oxidative stress during the imbibition phase [4]. These contrasting responses underscore the importance of copper form in determining early plant establishment.

Seedling growth traits exhibited substantial variability, with root length and fresh biomass showing significant treatment-dependent responses. CuO NPs at 25–50 ppm markedly enhanced root elongation and fresh weight, whereas  $\text{CuSO}_4$  treatments consistently suppressed these traits. Roots serve as the primary interface for nanoparticle uptake, and enhanced elongation likely reflects stimulation of meristematic activity and cell expansion under controlled Cu availability [31, 35]. From a mechanistic perspective, copper is an essential cofactor for enzymes involved in respiration, photosynthesis, and antioxidant defense, including cytochrome c oxidase, plastocyanin, and Cu/Zn-superoxide dismutase [30]. The gradual release of  $\text{Cu}^{2+}$  from CuO NPs likely enhanced these metabolic processes without exceeding toxicity thresholds. Conversely, ionic Cu from  $\text{CuSO}_4$  accumulates rapidly in root tissues, causing membrane damage and inhibiting elongation growth [2, 3]. Fresh weight responded more strongly than dry weight, suggesting that CuO NPs primarily promoted turgor-driven growth and metabolic activity during early development. Similar trends have been reported in tomato, and *Brassica* treated with CuO NPs [11, 20, 31].

## 2.3. Vegetative Growth Dynamics and Developmental Trajectories

Vegetative growth responses intensified with crop age, with CuO NPs at 25–50 ppm consistently producing the tallest plants at 15, 30, and 45 DAG. The increasing magnitude of treatment effects at later stages indicates that early nano-mediated advantages were maintained and amplified over time. Strong correlations among growth stages further confirm that early vegetative vigor establishes a developmental trajectory governing later performance. Similar persistence of nanoparticle-induced growth benefits has been reported for CuO and ZnO nanoparticles in vegetable crops [16, 43]. In contrast,  $\text{CuSO}_4$  treatments produced progressively lower growth with increasing concentration, reflecting limited translocation efficiency and localized ionic accumulation. Elevated ionic Cu is known to interfere with photosynthetic electron transport and auxin metabolism, leading to suppressed elongation growth [36]. Reproductive traits—pod number, pod length, and total yield—were highly sensitive to copper form and concentration. CuO NPs at 50 ppm produced the highest pod number and pod length, resulting in maximum yield per plant. Strong positive correlations between vegetative growth, pod traits, and yield indicate that yield enhancement is an integrated outcome of improved source–sink relationships.

Copper plays a central role in pollen viability, flower retention, and carbohydrate translocation to developing sinks [13]. Nanoparticle-mediated Cu delivery likely improved nutrient use efficiency and sustained Cu availability during reproductive stages, minimizing leaching losses and supporting continuous metabolic demand [44]. Comparable yield enhancements under CuO

NP treatments have been reported in okra, tomato, and *Brassica* species [17, 20, 31, 44]. Yield reductions observed under  $\text{CuSO}_4$  and higher extract concentrations reinforce the concept of dose-dependent phytotoxicity, where excessive ionic Cu or phytochemicals disrupt nutrient balance and reproductive development [4, 33]. Although fertilizer treatments improved growth relative to the control, they consistently underperformed compared with CuO NPs, highlighting the superior agronomic efficiency of nanoparticle-based copper delivery [19].

#### 2.4. Hormesis and Mechanistic Interpretation

The nonlinear response observed—stimulation at moderate CuO NP concentrations and inhibition at higher doses—reflects a classic hormetic pattern widely reported for metal oxide nanoparticles [6, 46]. At low doses, CuO NPs likely enhance redox signaling and enzyme activation, whereas excessive exposure overwhelms antioxidant defenses, leading to lipid peroxidation, protein oxidation, and impaired photosynthesis [2, 3]. The absence of severe phytotoxic symptoms at optimal CuO NP doses indicates that applied concentrations remained within the beneficial window. This aligns with reports of safe Cu accumulation within WHO limits following nanoparticle application in okra [44].

#### 2.5. Implications for Sustainable Nano-enabled Agriculture

Collectively, the findings demonstrate that green-synthesized CuO NPs represent an efficient, low-dose alternative to conventional copper fertilizers, delivering enhanced growth and yield while minimizing phytotoxic risks. Phytochemical capping derived from plant extracts improves nanoparticle stability, moderates  $\text{Cu}^{2+}$  release, and enhances biocompatibility, thereby supporting safe nutrient delivery [8, 28]. By integrating nanoparticle characterization with detailed biological performance across developmental stages, this study advances mechanistic understanding of CuO NP–plant interactions and supports the development of eco-safe, nano-enabled micronutrient strategies for climate-smart agriculture [41, 45].

### 3. Conclusion and Future Prospects

The present study demonstrates that green-synthesized CuO NPs applied through seed priming significantly enhance vegetative growth, reproductive traits, and yield of okra without adversely affecting germination. CuO NPs priming, particularly at 25–50 ppm, consistently outperformed ionic copper sources by improving seedling vigor, plant height, pod development, and total yield, highlighting the importance of copper form and controlled delivery. Strong correlations among early vegetative growth, pod traits, and yield further confirm that improved establishment plays a central role in determining final productivity.

From a future perspective, CuO NPs-based seed priming represents a promising nano-enabled approach for efficient micronutrient management in horticultural crops. Further studies should focus on long-term field validation, assessment of nanoparticle fate in soil–plant systems, and optimization of application rates under diverse agro-climatic conditions. Integration of this strategy into sustainable crop management frameworks could contribute to improved productivity while reducing reliance on conventional copper fertilizers.

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